

The University of Chicago

THE EFFECTS OF POPULATION
DENSITY UPON GROWTH, REPRO-
DUCTION, AND SURVIVAL OF
HYALELLA AZTECA

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

1939

By
JANET WILDER

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THE EFFECTS OF POPULATION DENSITY UPON GROWTH REPRODUCTION, AND SURVIVAL OF *HYALELLA AZTECA*

(Five figures)

JANET WILDER
University of Chicago

THE problems of the biotic relationships of like organisms have attracted much interest. Naturalists have reported with enthusiasm noteworthy aggregations, sexual behavior, and "social" manifestations. This work continues but with a more critical attitude. The less obvious mass relations, seen only by resultant effects, led to a dichotomy of interest, which in the future and to some extent at present (Park, 1939) will coalesce. One approach is that of the study of the whole population, its growth, maintenance, and decline or evolution (Chapman, 1928; Wright, 1931; Gause, 1934; Bodenheimer, 1938). The other is the study of physiological or behavioristic effects of population density upon the constituent organisms (Allee, 1931, 1934, 1938).

The physiological aspect is considered in the present study,¹ which attempts a demonstration of the effects of increasing the density of the population upon three fundamental processes: growth, reproduction, and survival. As an assay animal the common freshwater amphipod *Hyalella azteca* Saussure² (*H. knickerbockeri* Bate), Orchestiidae, was chosen. In the use of an aquatic arthropod the study is unique among researches comparable in nature to those of Pearl and of others to be cited later. The conditions of the experiments were variable, but equal volumes of food and water were used in all, irrespective of numbers of animals present. The following brief review of the literature is necessarily closely confined to the question studied and deals with recent studies on arthropods. The references given in the first paragraph include summaries of results on population studies of other forms. Earlier work by the same or different authors may be found by consultation of the literature referred to below.

Growth.—Body size (Warren, 1900) and rate of development (Banta, 1937) were retarded in Cladocera by crowding or its concomitant factors. Metamorphosis in the beetle *Tribolium confusum* was delayed, and body weight was reduced (Park, 1938) in relation to the density of the population and more directly to the degree of conditioning of the medium. On the other hand, evidence is present for an augmentative effect of increasing numbers of organisms. Development is accelerated in *Periplaneta orientalis* after the ninth instar, and the effect is apparent even in a density as low as 2 animals. However, the longest and heaviest animals were found among the isolated individuals, an effect attributed to increased disturbance by collision in higher densities (Landowski, 1938). Meal worms (*Tenebrio molitor*) raised 50 together in 60 cc. of meal consistently weighed more than did isolated larvae; even 150 animals in a similar volume maintained a higher weight than that of isolated ones until the sixtieth day (Michal, 1931). This was

I wish to express my gratitude to my adviser, Dr. W. C. Allee, for his interest in, and criticism of, this problem.

² To Mr. Clarence Shoemaker, assistant curator at the Smithsonian Institution, I am indebted for the identification of the animals.

attributed to an effect of increased temperature. Wardzinski (1938) believed this factor effective in the progressive acceleration of larval life in *Pieris brassicae* raised in densities ranging from 1 to 32 animals. The heaviest pupae of this butterfly occurred in populations of 2 or 4. Accelerated development in higher densities was found by Titschack (1936) and Mosebach-Pukowski (1937) in the clothes moth and *Vanessa*, respectively. Landowski (1938) cites Manujlowa, Kosmina, and Alpatov (1931), and Alpatov and Dorodnichaia (1932), as obtaining results indicating the inhibitory action of isolation on lepidopterans.

Reproduction.—Few studies on reproduction in regard to density in arthropods are at hand. The most complete concern *Drosophila melanogaster*, which showed a clearly inverse relationship to numbers of animals present (Pearl, 1932). In cladocerans reproduction was adversely affected by crowding (von Dehn, 1937). The retardative effect of crowding on the group fecundity of scales (*Lepidosaphes ulmi*) was due to the increased number of sterile females rather than to an effect upon the reproducing mothers (Smirnov and Polejaeff, 1934). On the other hand, crowding decreased fecundity in the moths *Arctia caja* and *Lymantria dispar* by lowering the maturation of ovarian eggs. This effect was attributed to malnutrition through disturbance by collision during feeding (Hofmann, 1933).

By counts of the growing populations the reproductive activity of flour beetles seems to be maximal at a population of intermediate density (Park, 1932; Maclagen, 1932; Maclagen and Dunn, 1936). Increased copulation frequency was directly shown to increase fecundity of *Tribolium* (Park, 1933) and may be operative in the greater population development of an initially intermediate density. However, this effect is offset to an increasing extent by the direct retardation of fecundity by increased conditioning of the medium (Park and Woollcott, 1937).

Survival.—A rough generalization on the effects of density upon survival may be attempted: under toxic conditions or those inimical by the absence of essential elements, survival is maximal at an intermediate density since the organisms or their products tend to ameliorate these conditions. Many instances are given by Allee (1931) and, more recently, by Allee and Wilder (1939). Careful analysis of death rate in *Drosophila* populations (Pearl, Miner, and Parker, 1927) demonstrated a similar effect, with the optimal density at 25–35 flies per 1-ounce bottle. However, survival is frequently inverse to the numbers of organisms present. This has been reported for cockroaches (Landowski, 1938) and for larval mortality of grain beetles (Maclagen and Dunn, 1936; Park, 1938).

MATERIAL AND METHODS

Hyalella azteca Saussure (*H. knickerbockeri* Bate) is a widely distributed amphipod. The specimens which were used came from a culture maintained for over ten years in the tanks of the department greenhouse, where they were accidentally seeded.

None of the research done upon amphipods has required the development of the standardized techniques necessary in quantitative studies on physiological effects. The most complete study of amphipods is that initiated by Sexton and continued by herself and others. This chiefly genetic study has been summarized by Sexton and Clark (1936). In their work the animals were kept in finger bowls and fed upon *Ulva* or dead leaves—a method altogether unsatisfactory for results dependent on the control of all factors, as far as possible, other than density of like organisms. In the search for greater control

much of the present work is exploratory and covers a rather wide field of observation in order to lay a basis for further analytical treatment.

The experimental procedure consisted in obtaining the needed number of newly released young within, if possible, 2-3 days. They were then counted out at random into 6- or 8-ounce, narrow-necked bottles, according to the planned population densities. The bottles had been filled with 100 cc. of water, to which were added either 2-inch lengths of *Elodea* or 2-4 drops of yeast. Usually the food and water was changed weekly until the end of the experiment. All experiments were conducted at room temperature, which was recorded by a thermograph (Table 1). Temperatures are given in degrees centigrade.

The above résumé needs further expansion. Several hundred females with brood in about the same stage were collected from the stock and kept in finger bowls. Shortly before the time of expected release of the young, the bowls were cleaned of all young then present; from that time on they were removed at frequent intervals and stored together in finger bowls for use in the experiment.

Elodea was first tried as food because it is one of the natural food plants of the amphipods (Embody, 1912). The long streamers were washed, stripped of their leaves, and scalded in hot tap water. The latter treatment was planned to kill associated organisms. The food was most unsatisfactory because of inequality along the length of the stem and from stem to stem. The hot-water treatment sometimes killed the plant and did not always kill the animals on the plant. For these reasons *Elodea* was abandoned after the third experiment.

Yeast was substituted, since it is a simple, easily measured food. Fleischman's yeast cake was made up with distilled water in the proportion of 1 gm. to 10 cc. The well-stirred stock suspension was fed to the populations by drop. The yeast in the population bottles was not itself growing but started to grow upon removal to a suitable nutrient solution. This took place even after the yeast had been 8 days in the clean water medium. No attempt at sterile precautions or at bacterial analyses has been made.

The initial densities seeded changed with the deaths of some of the animals. In order to maintain, as nearly as possible, the original densities, one or more bottles of a given population were eliminated, and the animals used to restore the numbers in the remaining bottles of that density. This could not be done in densities of 20, 50, and 100, where few bottles were available, especially in the two highest densities. Here the densities were allowed to drop but never to overlap. These populations were equalized by transference of animals between bottles of a given density.

The molted skins (exuviae) were collected at each time of change of water, or oftener, and preserved in 50 per cent alcohol. Later, magnified projection outlines of the mid-dorsal lines were drawn, and these were measured by calipers.³ It is necessary to use the exuviae for an accurate total-length measure because the animals are flexible, and the segments telescope within each other. The living whole animals, even when anesthetized, displayed all degrees of flexion. Since the exuviae were transparent, the overlap of segments might be seen, and the drawings were made to include this factor. The total lengths given, therefore, are those of the summated sclerites. The figures are the most accurate possible, but they are entirely dependent on a rough sampling of the population. As the animals became older, the validity of the representation suffered greatly because they molted less often, and they destroyed the exuviae. The latter was particularly

³ I wish to thank Miss Catherine Lutherman for her assistance in these measurements.

TABLE 1
EXPERIMENTAL CONDITIONS

Expt. Number	Initial Density	Number of Bottles	Remarks
<i>Elodea</i> I.....	2 5 10 25 50 100	25 10 5 4 2 1	Started 12/7/37, mean age of young: 2 days; temperature: mean 19°3, range 16°0-23°0; food and water: <i>Elodea</i> , tap water, both changed every other week; total number animals seeded: 450
<i>Elodea</i> II.....	1 10 20 50	50 5 2 1	Started 1/16/38, mean age of young: 2 days; temperature: mean 19°4, range 16°0-24°0; food and water: <i>Elodea</i> (living), charcoal-filtered tap water changed weekly; total number animals seeded: 190
<i>Elodea</i> III.....	1 8 20 50	50 5 2 1	Started 1/16/38, mean age of young: 6 days; temperature: mean 19°4, range 16°0-24°0; food and water: <i>Elodea</i> (living), charcoal-filtered tap water, never changed, poured through silk bolting cloth weekly; total number animals seeded: 180
Yeast I.....	1 2 4 10 20 50 100	50 50 25 20 5 2 1	Started 5/3/38, mean age of young: 1 day; temperature: mean 25°2, range 21°0-30°0; food and water: 2 drops yeast to 6/14, then 4 drops, filtered tap water, both changed weekly; total number animals seeded: 750
Yeast II.....	1 2 4 6 10 20	100 50 25 20 20 10	Started 8/3/38, mean age of young: 2 days; temperature: mean 28°0, range 24°0-31°3; food and water: 4 drops of yeast, filtered tap water, both changed weekly; total number animals seeded: 820
Yeast III.....	1 2 4 6 10 20	100 60 25 20 10 5	Started 12/7/39, mean age of young: 2 days; temperature: mean 21°0, range 19°5-22°0; food and water: 4 drops yeast, filtered tap water, both changed weekly; chlore-tone used after second week; total number animals seeded: 640; replacements on fourth day
Yeast IV.....	1 2 4 6 10 20	60 30 15 10 6 3	Started 2/22/39, mean age of young: 4 days; temperature: mean 20°9, range 19°5-22°0; food and water: 4 drops yeast, well water, both changed weekly; chlore-tone used after second week; total number animals seeded: 420
Yeast V.....	1 2 4 6 10 20 50	100 50 25 20 20 10 4	Started 2/26/39, mean age of young: 3 days; temperature: mean 20°9, range 18°6-24°0; food and water: 4 drops yeast, supplemented after 1 month of age by 2 inches <i>Elodea</i> stem, well water, both changed weekly; chlore-tone used after one month; total number animals seeded: 1,020
Yeast VI.....	1 2 4 6 10 20	80 40 20 14 8 4	Started 5/10/39, mean age of young: 4 days; temperature: mean 24°0, range 19°2-30°0; food and water: 4 drops yeast, well water, both changed weekly; chlore-tone used in first week; total number animals seeded: 564

true in a density of 10 or more. The measurement of size by exuviae has been used in a study of proportional growth in *Gammarus* (Sexton, 1924).

A second method was used after experiment Yeast II. All animals of a population were anesthetized in chloretone, and the head length was measured by ocular micrometer. The increased deaths from this method invalidated the survival record. However, the error in the growth analysis due to partial sampling was entirely obviated. To substantiate the results obtained in growth by measurements of body or head lengths the sum of the antennal segments of the first and second antennae of one side was usually recorded also ("antennal segment number").

Fecundity was demonstrated by removal and preservation of the females carrying brood. They were later measured, and the eggs stripped from the pouch and counted. Only three experiments lasted long enough with a sufficiently high survival to provide such data.

By counting the animals when the water was changed, the number surviving was easily obtained. Survival has been followed only until the time of female maturity, to avoid the necessity of a correction factor for the removed females. Also survival records have not been used after anesthesia by chloretone.

EXPERIMENTAL RESULTS

GROWTH

Isolated controls.—The broods of 10 females were isolated, on May 4, 1938, 1 young per 100 cc. of filtered lake water with 1 drop of yeast. Initially, 107 first-instar animals were obtained. The water and food was changed weekly; at the thirty-eighth day the

TABLE 2*

GROWTH OF ISOLATED *H. azteca* FED UPON YEAST FROM THE FIRST TO THE TENTH INSTAR

Instar	Days of Age at Given Molt	Duration of Instar (Days)	Body Length (Mm.)	Increments of Growth (Mm.)	Percentage Head to Total Length	Number of Antennal Segments of One Side
1.	5.2±0.1	5.2±0.1	1.28±0.01		13.6	13.0±0.1
2.	10.7±0.2	5.4±0.3	1.53±0.02	0.24±0.02	12.5	13.6±0.1
3.	16.6±0.3	5.9±0.2	1.83±0.03	0.31±0.02	12.0	14.4±0.2
4.	22.6±0.4	6.1±0.3	2.17±0.04	0.33±0.03	11.2	15.2±0.2
5.	28.9±0.4	6.1±0.3	2.48±0.05	0.31±0.03	10.9	16.6±0.2
6.	34.5±0.6	5.9±0.3	2.83±0.04	0.35±0.02	10.4	17.6±0.2
7.	41.9±1.1	7.6±0.3	3.23±0.06	0.38±0.02	10.1	18.9±0.3
8.	49.6±1.2	8.8±0.4	3.65±0.06	0.42±0.02	10.0	19.9±0.3
9.	58.0±0.7	9.3±0.4	4.00±0.08	0.38±0.03	9.5	21.1±0.3
10.	65.2±1.2	9.1±0.4	4.40±0.11	0.37±0.04	9.4	22.1±0.7

* Means and standard errors are calculated from 72 animals providing data on time and age, of which 42 were used in the calculation of morphological values.

food was increased to 2 drops of yeast, and on the seventy-second day to 4 drops. This experiment was run synchronously with Yeast I; therefore the conditions are comparable to those of that experiment. The bottles were observed daily, molts recorded, and exuviae preserved for later measurement. Only the data pertinent to this problem will be discussed. Deaths reduced the number of animals to 73, and of these only 42 series of exuviae were sufficiently complete for growth records. Table 2 presents the mean values and their standard errors.

TABLE 3
MEAN BODY LENGTH (MM.) OF ANIMALS IN POPULATIONS
FED UPON *Elodea*

(The lower half of each table presents the significance [*P*]
of the difference between the designated populations)

A. *Elodea* I

AGE IN DAYS	INITIAL POPULATION DENSITY					
	2	5	10	25	50	100
16.....	2.02	2.10	2.19	2.18	2.11	1.84
40.....	4.27	3.75	3.79	3.83	3.33	2.51
45.....	5.32	4.70	4.48	4.25	3.72	2.90
52.....	5.79	5.64	5.41	4.47	3.78	2.95

AGE IN DAYS	COMPARED POPULATION DENSITIES					
	2:5	5:10	10:25	25:50	50:100	2:10
16.....	0.44*	0.33	0.84	0.16	0.008	0.11
40.....	0.04	0.22	0.02	0.001	0.0001	
45.....	0.03	0.38	0.27	0.02	0.002	
52.....	0.63	0.49	0.01	0.06	0.010	

B. *Elodea* II

AGE IN DAYS	INITIAL POPULATION DENSITY			
	1	10	20	50
9.....	1.39	1.38	1.34	1.23
16.....	1.91	1.76	1.80	1.72
23.....	2.32	2.44	2.25	1.87
30.....	3.16	2.97		
72†.....	5.18	4.27	3.69	3.04

AGE IN DAYS	COMPARED POPULATION DENSITIES		
	1:10	10:20	20:50
9.....	0.02	0.42	0.11
16.....	0.13	0.13	0.06
23.....	0.62	0.39	0.12
30.....	0.43		
72.....	0.0001	0.09	0.15

* The values are probabilities (*P*). A *P* of 0.05 (5 chances in 100 of getting a similar difference by "chance") is regarded as the upper limit of significance. The smaller the decimal the greater is the significance.

† Lengths are of all survivors. Sexes approximately equally represented.

The changes from instar to instar were quantitative; no qualitative criteria were found. The antennal segment number for each instar was highly variable, and there are indications that it cannot be regarded as a valid index of instar. Sexual differentiation also was gradual. No earlier than the seventh instar (about 40 days), often much later, the males might be distinguished by the heterogonic development of the large second gnathopods. On the average, the males in the experiments were larger after maturity than were the females. In the experimental results presented later the percentage of developed males is given, when possible, to indicate whether disproportion of the sexes among the populations is correlated with mean size differential. The first molt occurred at about 5 days of age (Table 2). In the experiments body length was first measured at least 1 week after the start of the experiment, at which time the animals would usually be between 8 and 11 days of age. Therefore, the first recorded mean size covers a mixture of first- and second-instar animals. Table 2 shows also the percentage relation of head to total length, since these two measures have been utilized in the experimental results.

TABLE 4*
GROWTH IN POPULATIONS FED UPON YEAST IN EXPERIMENTS I-VI

AGE IN DAYS	INITIAL POPULATION DENSITY					
	1	2	4	6	10	20
10.4.....	1.67	1.73	1.76	1.78	1.77	1.78
17.6.....	2.41	2.47	2.55	2.52	2.43	2.56
26.2.....	2.96	3.00	3.02	2.98	2.84	2.84
33.7.....	3.83	3.96	3.89	3.56	3.44	3.32
44.0.....	4.51	4.29	4.41	4.23	4.13	3.95

* The values are means of total length and head length $\times 10$ (mm.). The boldface figures are maximal for each age.

Growth of animals in populations fed on Elodea.—Of the three experiments in which *Elodea* alone was used as food, only two furnished data upon the growth of the animals. In the third experiment, in which the water was never changed, insufficient numbers of molted skins were obtained, either because the animals molted rarely or at once destroyed them. From Table 3 it may be seen that, on the whole, mean body size was inversely related to the density of the population. The maximal size at density 10—16 days—in *Elodea* I was not significant, but the indication is of interest in view of later results. The significance of the difference between densities increased with age (Table 3, A, 16 days compared to 40 days) and with increasing density (25:50, compared to 50:100). In *Elodea* II none of the observed differences is significant until age 72 days, at which time the isolated individuals were significantly larger than those in population 10.

Growth of animals in populations fed upon yeast.—When compared to the results of *Elodea* feeding, animals in populations fed upon yeast show another effect. Table 4 presents an average of the six experiments at the ages and densities which are comparable. Population density 6 has been interpolated for Yeast I. The results on head length in Yeast III through VI have been multiplied by 10 to weight them nearly equal to body lengths. The table shows a shift of mean maximum size from a high density to lower den-

sities with advancing age. The results of separate experiments bring this point out more clearly. Figure 1 presents the results of the first yeast experiment. In this experiment only 2 drops of the stock yeast suspension were added to the 100 cc. of filtered lake water. After the experiment was in progress, it became apparent that this was not sufficient, since population 10 cleaned it out of the water before the week between feedings was complete. However, such feeding was continued until the maximal growth of population

1 was well established (Fig. 1, 43 days). On the forty-fifth day bi-weekly feeding was started, making a total of 4 drops per week to each population, an amount used in all later experiments. Only the densities through 20 have been used in the figure. Density 100 provided practically no exuviae for length measurements; the density of 50 and 20 gave data through the twenty-fifth day. The failure of data for these higher densities is largely due to the destruction of the exuviae and, possibly, to less frequent molting. Consequently, the few molted skins obtained were an unreliable sample. However, the trend of the higher densities is indicated at the eighty-eighth day by the result in density 10 and 20. At this age all remaining males were preserved and later measured so that the values are derived from the whole male population. The populations did not invariably fit the trend. Pearl and Parker (1922) write that, owing to accidents relative to the smallness of numbers at certain populations, their populations did not "fall in line." The discussion of survival will show this more clearly in the amphipod populations (Fig. 5).

During the first 2 weeks of age the animals of population 20 were significantly larger than those isolated ($P = 0.017$ and 0.002 for 8 and 15 days of age). Thereafter, body length tended to become inverse to the population density, although the difference between densities of 1 and 2 was not significant. After the food was doubled in quantity, an insignificant ($P =$

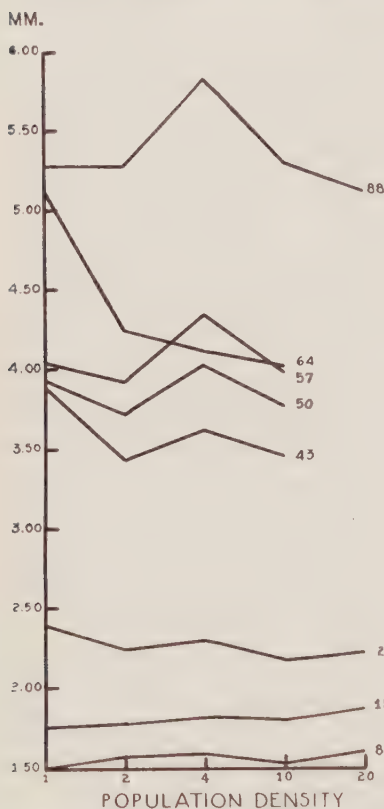


FIG. 1.—Mean body length of populations in Yeast I. Age in days to the right of each curve.

0.271) maximum occurred at density 4 through the fifty-seventh day. Failure of significance is related to the small number of exuviae obtained in any population and their great variability in length. At the sixty-fourth day the isolated animals were on the average of greatest body length. The difference between densities 1 and 4 is significant ($P = 0.028$), despite the fact that the 24 isolated animals for that day were represented by 6 exuviae only. The sampled animals of population 4 were of smaller size in general than on the preceding date (57 days). Probably those molted skins were from females, which at sexual maturity show the inverse relationship of body length to density (Fig. 4, C) and which at that time, in general, are smaller than the males. Males, on the

other hand, in the three experiments (Yeast I, IV, V) which continued long enough for this measure, invariably were of greatest mean length in a density of 4 (Fig. 3). The latter observation was shown in Yeast I by the mean body lengths of the preserved males at the eighty-eighth day of age. This curve strongly suggests those of the three ages prior to the sixty-fourth day of age. Counts of the developed males made on the sixty-eighth day showed the highest proportion of that sex in the population density of 4; the percentages for populations 1-50 were, respectively, 41.2, 54.0, 64.9, 51.2, 48.0, and 14.6.

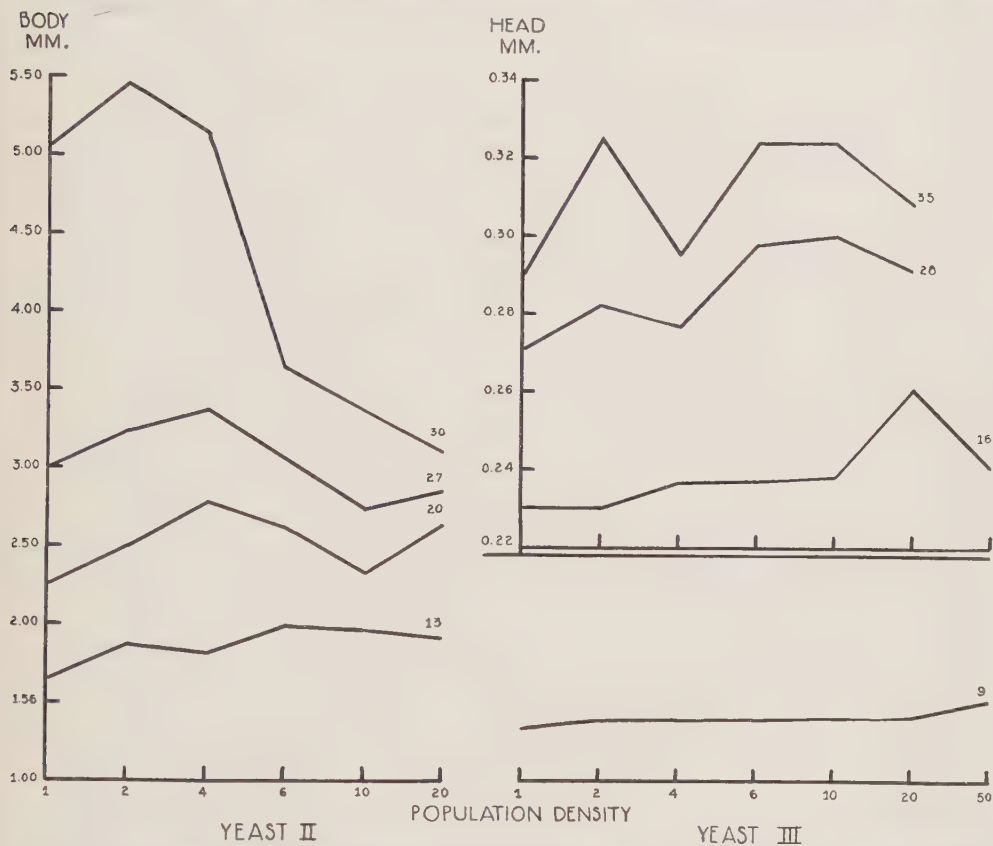


FIG. 2.—Mean body or head length of populations fed upon yeast. Age in days to the right of each curve

These figures are not basic sex ratios, which are approximately 1:1, because the females which had shed their eggs had been removed from the population and because the immature males are practically indistinguishable from females without brood or mature ovaries. There is, unquestionably, delay of sexual maturity in populations of 50 or more. The percentages given above indicate that the preponderance of developed males may have occasioned the peak of size at population 4 from the forty-third day.

The next two experiments were planned to demonstrate the growth of the animals in the populations prior to sexual maturity, in order to obviate the complication introduced by the presence of mature males and females. Both are presented in Figure 2. The sec-

ond type of length measure was instituted in the second week of Yeast III (16 days of age); all animals were anesthetized, and the head lengths measured by an ocular micrometer. The values are, therefore, means of the entire population. The same is true of the thirtieth day in Yeast II, for the values are derived from measures of the whole preserved animals. The results show a shift of mean maximal size from a higher density to a lower with advancing age. In Yeast I (Fig. 1) the isolated animals were maximal in size on the twenty-fifth day; with double the food the shift in Yeast II and Yeast III had fallen to the density of 2 at the thirtieth and thirty-fifth days, respectively. The delayed drop of the maximum may be correlated with the increased quantity of food. Yeast III started on the ninth day with maximal exuvial length in population 50, but in Yeast II on the thirteenth day the population 6 is the largest. The difference between the two experiments is not explainable by changes in technique, but it does not seem unreasonable, since the former was started in December and the latter in August.

In Yeast II the population of mean maximal size was at no time significantly bigger than the isolated animals. However, in Yeast III significance obtained at all but the last age; the P values, the maximum compared with the isolated animals at the four ages, are, respectively, 0.003, 0.0001, 0.016, and 0.093.

The preceding three experiments have given reasonably comparable results. The water used in all three was charcoal-filtered Lake Michigan (city) water from the laboratory tap. In order to see whether the drift of maximal growth was related to the type of water used, a change was made in the next three experiments (Yeast IV, V, VI) to Whitman well water. Quantity of food was maintained as in Yeast II and Yeast III at 4 drops per week.

Yeast IV has been chosen to be presented in complete tabular form (Table 5), since this experiment included exuvial length, head length, and antennal segment number through the sixtieth day.

The outstanding feature of Yeast II and Yeast III—namely, the shift of mean maximal length from a high density to a lower—occurred again, although somewhat less regularly. With great individual variation, as has been previously mentioned, sexual maturity starts after the fifth week, so that the maximal size of density 4, from the thirty-ninth day, as in Yeast I, may be influenced by the presence of the males. As before, the percentage of developed males was greatest among the animals of density 4. These percentages are 41.6, 35.7, 46.1, 34.8, 41.2, and 35.0 for population densities 1–20, respectively. Also the males of density 4 are largest, although not significantly (Table 5, 60 days; Fig. 3).

The absence of exuvial values for populations 2 and 10 on the thirty-second day of age is due to the fact that no molted skins represented those populations. The destruction of "molts" throughout all populations was so great thereafter that collection of them was abandoned. The data on head length, by their freedom from sampling error, are more reliable, but both measurements show in part a significantly greater size of the animals in the higher densities at an early age. The animals represented on a given date by molts were of an earlier instar than the same animals within the whole population valued by head length on the same date. In C with exceptions the number of antennal segments tends to support the growth effect presented in A on the basis of length.

Yeast V was designed originally for the study of survival and fecundity only. It was very difficult to see the eggs within the brood pouch in yeast-fed females because the yolk remains practically colorless. The addition of *Elodea* to the 4 drops of yeast after

TABLE 5

GROWTH OF ANIMALS IN YEAST IV

A. MEAN BODY (*a*) AND HEAD (*b*) LENGTHS IN MILLIMETERS

AGE IN DAYS	INITIAL POPULATION DENSITY					
	1	2	4	6	10	20
11 a*	1.56	1.50	1.67	1.65	1.67	1.66
18 a.	1.06	1.93	1.97	1.84	1.86	2.04
b†	0.27	0.27	0.28	0.28	0.28	0.28
25 a.	2.43	2.45	2.54	2.43	2.42	2.43
b.	0.31	0.31	0.32	0.31	0.30	0.31
32 a.	2.98		3.26	2.89		2.90
b.	0.36	0.38	0.37	0.35	0.34	0.34
39 b.	0.40	0.41	0.43	0.39	0.39	0.38
46 b.	0.45	0.44	0.46	0.43	0.44	0.40
53 b.	0.48	0.47	0.48	0.45	0.47	0.46
60 b‡	0.51	0.52	0.53	0.52	0.51	0.46

B. SIGNIFICANCE (*P*) OF DIFFERENCE BETWEEN ABOVE MEAN LENGTHS

AGE IN DAYS	COMPARED POPULATION DENSITIES								
	1:2	2:4	4:6	6:10	10:20	1:4	1:6	1:10	1:20
11 a.....	0.42	0.016	0.37	0.62	0.92	0.004	0.021	0.021	0.004
18 a.....	0.69	0.37	0.004	0.48	0.009	0.84	0.09	0.23	0.33
b.....	0.84	0.09	0.92	0.32	0.37		0.02		0.036
25 a.....	0.84	0.48	0.19	0.76	0.92	0.32			
b.....		0.48	0.19	0.13	0.11	0.48			
32 a.....			0.13			0.23			
b.....	0.028	0.62	0.06	0.27	0.55				
39 b.....	0.48	0.31	0.009	0.48	0.32	0.09			
46 b.....	0.84	0.42	0.07	0.32	0.0001	0.61			
53 b.....	0.56	0.55	0.19	0.48	0.84				
60 b.....	0.62	0.84	0.69	0.42	0.001	0.54			

C. ANTENNAL SEGMENT NUMBERS

AGE IN DAYS	INITIAL POPULATION DENSITY					
	1	2	4	6	10	20
11 a*	13.0	13.0	13.0	13.0	13.0	13.1
18 a.	14.8	14.3	14.6	14.6	14.6	14.8
b†	15.7	15.4	15.9	15.7	15.5	15.7
25 a.	15.7	16.1	16.3	16.2	15.4	16.3
b.	16.9	17.1	17.2	17.0	17.0	17.2
32 a.	19.0		18.6	16.8		17.6
b.	19.3	19.5	19.4	18.4	18.3	17.9
39 b.	20.4	21.1	21.2	20.4	19.8	19.4
46 b.	22.2	22.1	22.2	22.7	21.3	20.3
53 b.	23.4	22.7	22.8	21.9	22.1	21.1
60 b‡	23.8	23.2	24.4	22.3	22.8	21.6

* *a* = body length or antennal segment number derived from exuviae.† *b* = head length or antennal segment number from anesthetized animals, except at 60 days when preserved males used.

‡ Each population represented only by males.

the first month was planned to obtain green-colored eggs. It was hoped that by starting a large experiment, not using chloretoone, and feeding in this way, a great number of fecund females would be obtained. After 1 month of age the survival was so high that it was decided to risk the deaths by chloretoone and take a measure of growth. The readings after the first were of animals fed yeast and *Elodea*.

At the age of 34 days the animals in density 4 were of significantly ($P = 0.028$) longer average head length (0.38 mm.) than those in density 2 (0.36 mm.), which did not differ significantly ($P = 0.76$) from isolated animals (0.36 mm.). The higher populations showed a drop progressively, starting with 6 (0.36 mm.). At 48 days of age, 14 days after the first *Elodea* feeding, the isolated animals were of insignificantly maximal size ($P = 0.33$ when compared with density 2). As before, the males of density 4, on the sixty-second and seventy-sixth day were maximal in head length and antennal number

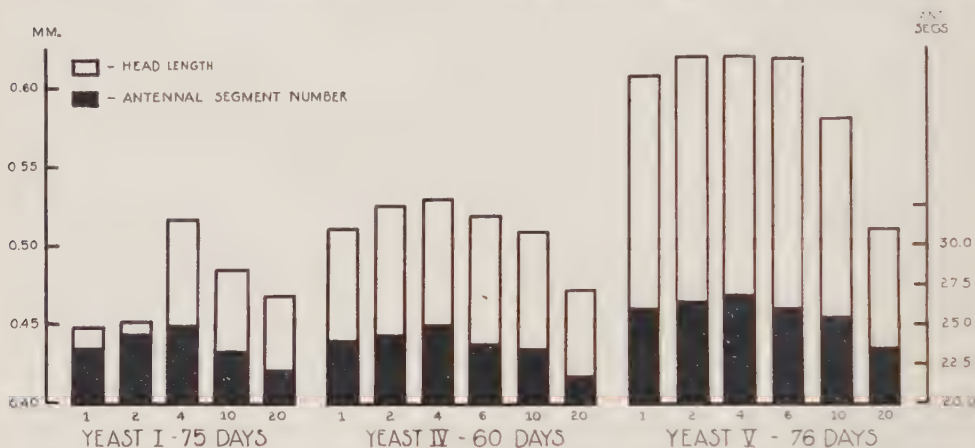


FIG. 3.—Head lengths (left ordinates) and antennal segment numbers (right ordinates) of sexually mature males fed upon yeast. Abscissae, population density.

(Fig. 3). This difference was not significant ($P = 0.10$). The results on mature males in Yeast I, IV, and V are presented in Figure 3. The head lengths of these males are not significantly greater than those of sparser populations except in Yeast I, where the mean head size of density 4 has a P value of 0.007 over that of density 2. The body length of sexually mature females, on the other hand, is inverse to density at the mean age of first oviposition (Fig. 4, C).

Yeast VI was started at the same time as Yeast V and was designed purely to recheck the growth effect. Since no survival data were required, chloretoone anesthesia was begun at the end of the first week (11 days of age). Either for this reason or for some other, the results were atypical in comparison with the other yeast experiments. At 11 days of age maximal head size was obtained in the density of 4, but thereafter the mean greatest head length was displayed by 2, 1 and 2, 2, and 4 in that order in the five determinations covering the 30 days of the experiment. Neither do the antennal segment numbers correlate well with head size. None of the maxima is significantly greater than the mean value from sparser populations.

When measurements were made on the eleventh day one-half of the animals (40)

were anesthetized and measured. It would have been impossible to measure 80 animals in 6 densities within one day, hence the number was halved. This may have contributed to the atypical results, but two technically interesting facts emerged. The deaths were much greater the week after the use of chloretone on those animals, 18.1 per cent as compared to 8.0 ($P = 0.001$), and they were of smaller mean head size; the difference of 0.015 mm. is significant ($P = 0.0004$).

In conclusion, from the results on growth in relation to the density of the population two effects have emerged. When the animals were fed upon *Elodea*, growth was inversely related to the density of the population in city water with and without chlorine. In the former case a slight indication of a maximum in a density of 10 was obtained at the sixteenth day, but this was not significant. However, when yeast was used as food and the water was either dechlorinated city water or well water, early growth was greatest at a high density; but with increasing age animals in lower densities were, on the average, larger. This was a fairly regular shift. The above trend recurred in five experiments, and frequently a statistically significant difference was observed. This was especially true when the compared densities were several steps apart in the experimental gradation. There is an indication that increased quantity of yeast will shift the maximum toward higher densities. The mature males of mean greatest size occurred without exception in the population density of 4. The isolated mature females, on the other hand, at the average age of shedding of first eggs were of largest size.

REPRODUCTION

Behavior.—A mature female oviposits about every 8 days. The eggs are incubated for about 6 days, and shortly thereafter the hatched young leave the pouch. Toward the end of incubation a male carries the female by hooking the dactyl of the first (small) gnathopods under the lateral edges of the second thoracic segment. Amplexus may last only 1 day prior to oviposition, or it may continue much longer if the female is skipping one or more ovulations; a duration of 28 days continuous amplexus has been observed. However, it apparently never occurs during the first 3 days of incubation. On the average it may be said that the male carries the female for about 4 days. During this time release of the brood of the previous mating, molting of the female, insemination, and oviposition occur.

In *H. azteca* the male does not necessarily desert the female at molting. In fact, he may apparently assist the molt (Sexton and Matthews, 1913) by arching up over the female's back and scraping the integument with the uropods. Afterward, insemination occurs during a frequently repeated palpation of the base of the pouch. During this action the male shifts his grasp on the female in order to lie diagonally crosswise and to curve his abdomen beneath that of the female. Oviposition usually takes place within 12 hours of molting, and further attempts at copulation are vigorously resisted, although the male continues for some hours to carry the female.

Female amphipods of many kinds are said never to shed eggs unless carried at some time in the cycle by a male. Embury (1912) cites this to be the case for all amphipods which he studied, including *H. knickerbockeri* (a synonym of *H. azteca*). However, in the present experiments females isolated from the first instar shed eggs. Sexton (1924) reports failure of shedding eggs by isolated females of *Gammarus chevreuxi* and *G. pulex*, but not in *G. locusta*. There were, however, indications of a larger brood size in the latter females when mated. The sterile eggs do not develop and are cast within a few days.

While compiling data upon life-history, counts of the broods of 346 stock females were made at various times. On the average 5.2 eggs were produced per stock female. Two tests of the correlation between brood size and female size (using head length as an index) were made. Forty-six animals measured on July 12, 1937, showed an average head length of 0.510 mm. and a brood size of 4.5 eggs. The correlation is significant ($r = 0.627 \pm 0.089$).⁴ Seventy-four others (July, 1939) gave 0.493 mm. head length and 4.0 eggs per brood ($r = 0.537 \pm 0.083$). Since amphipods increase in size at each molt, even after sexual maturity, it is impossible to state with these measurements whether the correlation is more directly related to female size or to female age.

An increase in fecundity with increasing age, presumably up to a certain limit, has been reported for amphipods (O'Brien and Yarnold, 1937; Embury, 1912; Sexton and Matthews, 1913). However, these authors did not attempt to segregate the factors of age and body size.

The effect of density.—Two hundred and sixty-eight females from three experiments provided the data to be discussed and represent three conditions of food: *Elodea* (*Elodea* I); yeast supplemented after 1 month by *Elodea* (Yeast V); and yeast alone (Yeast I). The results are graphically presented in Figure 4. In *Elodea* I densities 5 and 25 have been plotted with 4 and 20. Density 100 is omitted from the curve of *Elodea* I because at the time of last observation (70 days) only 2 females had shed eggs. Omitted also are densities 100 and 50 from Yeast I, since no matured females represented the first and only one the second at the seventy-seventh or last day of record. Otherwise, all densities of the three experiments are represented by from 5 to 30 females. The higher numbers occurred in densities 10–25, probably correlated with the higher survival (Fig. 5). The lack of females from densities 100 and 50, however, was not associated with a low survival but resulted from an actual retardation or inhibition of maturity. This is presaged by the retarded age at sexual maturity of the animals in density 20 and also in density 50 where represented (Fig. 4, B).

By analysis of the combined data from populations 2, 4, 10, and 20, which are densities present in all three experiments, a few observations pertinent to the relation of reproduction and diet may be drawn. The differences mentioned below are highly significant ($P < 0.00001$), unless otherwise stated. The sexually active females fed upon *Elodea* throughout life were larger (5.62 mm.) than those fed upon yeast for all (4.50 mm.) or for the first month of life (4.47 mm.). The addition of *Elodea* after 1 month had no significant effect on body size when these animals are compared with those fed upon yeast continually ($P = 0.19$).

The fecundity of the *Elodea*-fed females (8.0 eggs per brood) was much higher than that of the yeast-fed (4.7 eggs per brood). The addition of *Elodea* to a yeast diet significantly increased fecundity (5.0 eggs, $P = 0.005$), but the fecundity from the constant *Elodea* diet was even higher ($P = 0.0001$).

As the yeast-fed animals were retarded in body length and fecundity, so also were they retarded in age at the shedding of the first eggs (60.7 days old) compared to that of the *Elodea*-fed animals (52.0 days). The most rapid development occurred, however, among the animals fed upon both (47.7 days).

In such comparisons it is necessary to consider temperature. The maximal response of the *Elodea*-fed females occurred in a midwinter experiment ($10^{\circ}.3$ average temperature), the retarded yeast-fed animals were living under a mean temperature of $25^{\circ}.2$ in

⁴ An r value which is greater than twice its standard error has statistical significance.

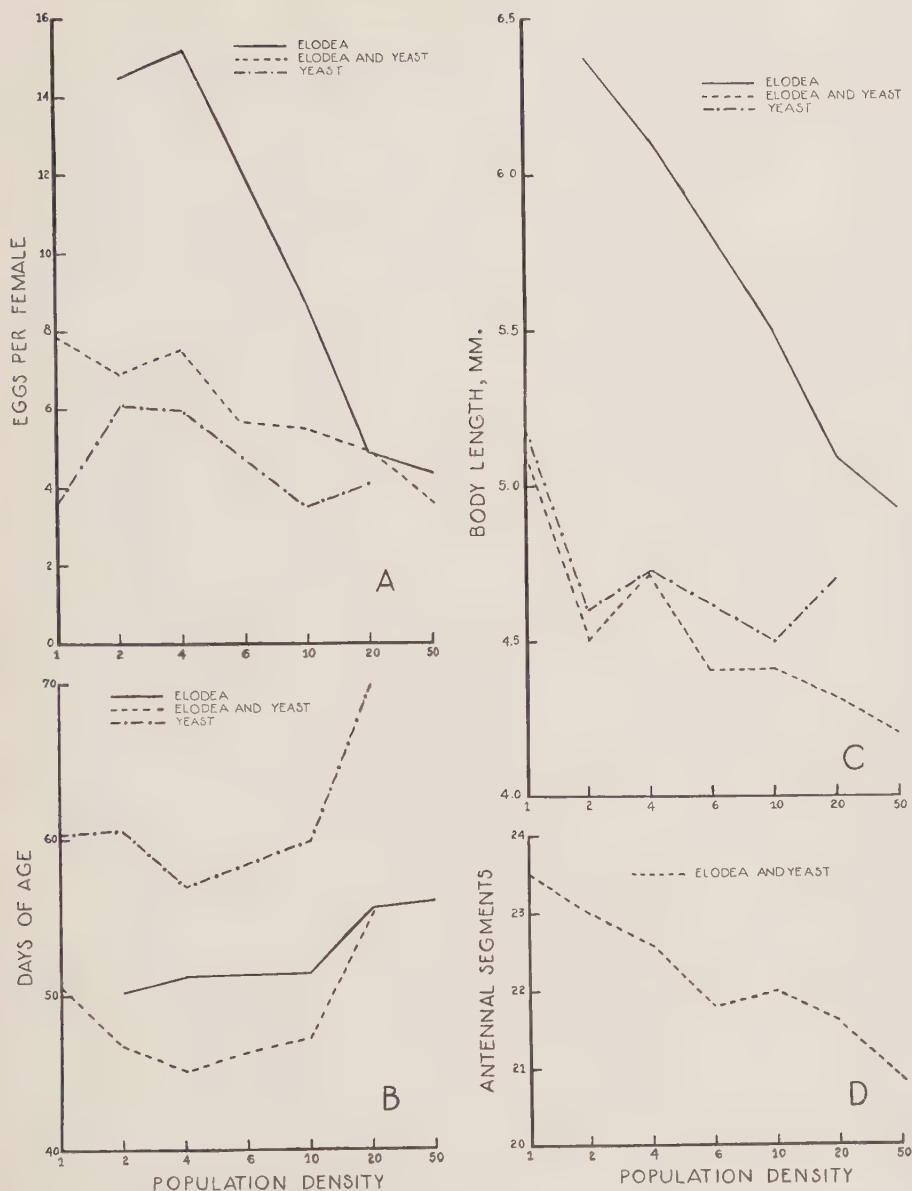


FIG. 4.—Mean fecundity (A), age at first oviposition (B), body length (C), and antennal segment numbers (D) of females in Elodea I (—), Yeast V (---), and Yeast I (- · -).

midsummer, and the yeast and *Elodea* feeding was conducted in the spring with an average temperature of 20° 9 (Table 1). Therefore, the difference in rate of development in respect to *Elodea* and to yeast is not in accord with the usual temperature effect. This effect was seen in stock females, of which the summer animals had an interovulatory period of 7.2 days (July) and in winter 12.2 days (January). Temperature may, however, have been effective in inducing significantly more rapid sexual development in the yeast-fed and *Elodea*-fed animals compared to that under constant *Elodea* feeding.

In relation to the effect of density upon reproductive activity it was found that the mean body size of females at the time of first oviposition was roughly inverse to the density (Fig. 4, C). The *Elodea*-fed animals showed the trend most clearly, but even on a yeast diet the difference between isolated and paired animals was significant ($P=0.024$). The antennal segment number, taken only in the yeast-*Elodea* experiment, was also inverse to density (Fig. 4, D). If antennal segment number is an index to instar, it is necessary to say that the 24 females in a density of 50 reached sexual activity at an earlier instar than the isolated animals, for the former possess on the average 20.0 segments and the latter 23.5. By reference to Table 2, the observed 20.0 segments would correspond to about instar 9, and the higher figure to instar 11 or 12. This result throws grave doubt on the validity of correlating antennal segments and instar stage, since a density as high as 50 animals, at least at the age of sexual maturity, has retarded all processes here studied.

Age at first sexual activity (Fig. 4, B) showed a tendency to increase with increasing density when the animals are fed upon *Elodea*. However, in the experiments in which yeast was used, sexual maturity on the average occurred most rapidly in population 4, the same density in which males had the largest size (Fig. 3). The accelerated maturity of density 4 over density 1 is significant in Yeast V ($P=0.003$).

The relation of fecundity to density is made difficult by the absence of a density 1 in *Elodea* I and the paucity of isolated females in Yeast I. In the latter experiment, however, the 12 paired females shed significantly more eggs than did the 5 isolated (Fig. 4, A; $P=0.010$). This is contrary to the correlation in stock females between body and brood size, for these more fecund paired females were smaller in size than the less fecund isolated animals. In *Elodea* I the increase in brood size of density 4 as compared to density 2 is insignificant ($P=0.84$), but the drop from density 4 to density 10 approaches significance ($P=0.09$). In consideration of the three curves (Fig. 4, A), with an increase in density above 4, an inverse relationship appeared between fecundity and density. Prior to that density the numbers present seemed to have little effect, at least when the natural food *Elodea* is included in the diet.

In conclusion of the section on reproductive activity it may be said that *Elodea* fed constantly from birth increased the size of the females when adult, the fecundity, and the rate of sexual development as compared to the effect of a yeast diet. The latter two aspects were also accelerated when *Elodea* was added to a yeast diet.

The females showed in general that body length and antennal segment number were in inverse relation to density. The mean age at first oviposition is youngest in population 4 of the animals fed yeast or those fed yeast and *Elodea*. The number of eggs per brood, apart from density 1, is not significantly affected by density, except on a yeast diet, until 6 or more amphipods are present. However, in the present state of our knowledge these indications cannot be taken too seriously.

SURVIVAL

General.—In order to obtain a basis for statistical analysis of survival, the time taken to reach 50 per cent survival was calculated for each population density of the eight experiments. This was converted to a percentage of the total time used by all densities of each experiment. On this percentage basis densities 1 and 2 were compared with densities 10 and 20; the latter were found to survive significantly longer ($P = 0.001$). Likewise, densities 10 through 100 were significantly more resistant than isolated animals and those of lower densities ($P = 0.005$).

The above generalization indicates the greater ability to survive of the individuals living in the higher densities. This generalization is substantiated in the results of the separate experiments which are presented in survivorship curves (Fig. 5). In these curves time—that is, age in days—is plotted on the abscissae and the number living on the ordinates. The latter figure is a percentage, since the number has been corrected to an initial seeding of 100 animals. In order to avoid confusion by the interlacing of curves only 5 population densities are given for each experiment. In *Elodea* II and III only 4 population densities were used (Table 1); therefore, those experiments are presented completely.

Populations fed upon Elodea.—Three facts were indicated by the first experiment (Fig. 5, *Elodea* I), which were supported by later observations. First, the animals in a very high density (50) survived best early in life, but, as they grew older or increased in size, maximal survival appeared and was maintained in an intermediate density (25). Secondly, populations did not always follow the general trend (density 5). Lastly, the decrease in numbers living was great at first among the animals of low densities and only later in the high densities; compare the curve of the paired animals and that of the density of 50.

Elodea II and III were alike in all particulars except that in *Elodea* III the water was never changed but merely passed through silk bolting cloth weekly. Both experiments were conducted at the same time. As in *Elodea* I, maximal survival shifted in *Elodea* II from a density of 50, and, since density 20 was out of line, density 10 survived maximally from the thirty-sixth day until the end of the counts on the fiftieth day. In *Elodea* III the animals of population 8 lived best from the beginning until the end. The greater death rate, in this experiment, of populations 20 and 50 was apparently correlated with the heavily conditioned, unchanged water. This result is atypical compared to those of the other experiments.

Populations fed upon yeast.—The type of food appeared to have no effect on the influence of density upon survival. In Yeast I (Fig. 5), as in *Elodea* I and II, a dense population (100) survived maximally at first, until the nineteenth day of age. Thereafter, density 20 best maintained its numbers until the end of the counts (32 days). After its initial maximal survival, the death rate in population 100 was so great that only 42 per cent were alive at the end, as compared to 56 per cent of the single animals or 63 per cent of density 50 (not graphed). In line with earlier results the deaths were initially most numerous among the isolated animals, after that the survivors maintained their numbers.

In Yeast II and III the death rate through all populations was very high for some unknown reason. However, in Yeast II, the maximal survival of an intermediate density (10) was demonstrated again, and also the minimal resistance of the isolated animals for at least the first month of age. Yeast III provided the only exception to the observation that isolated animals never survived maximally. In fact, so irregular was the survival of the populations that it was difficult to see a trend had it not been for preceding and fol-

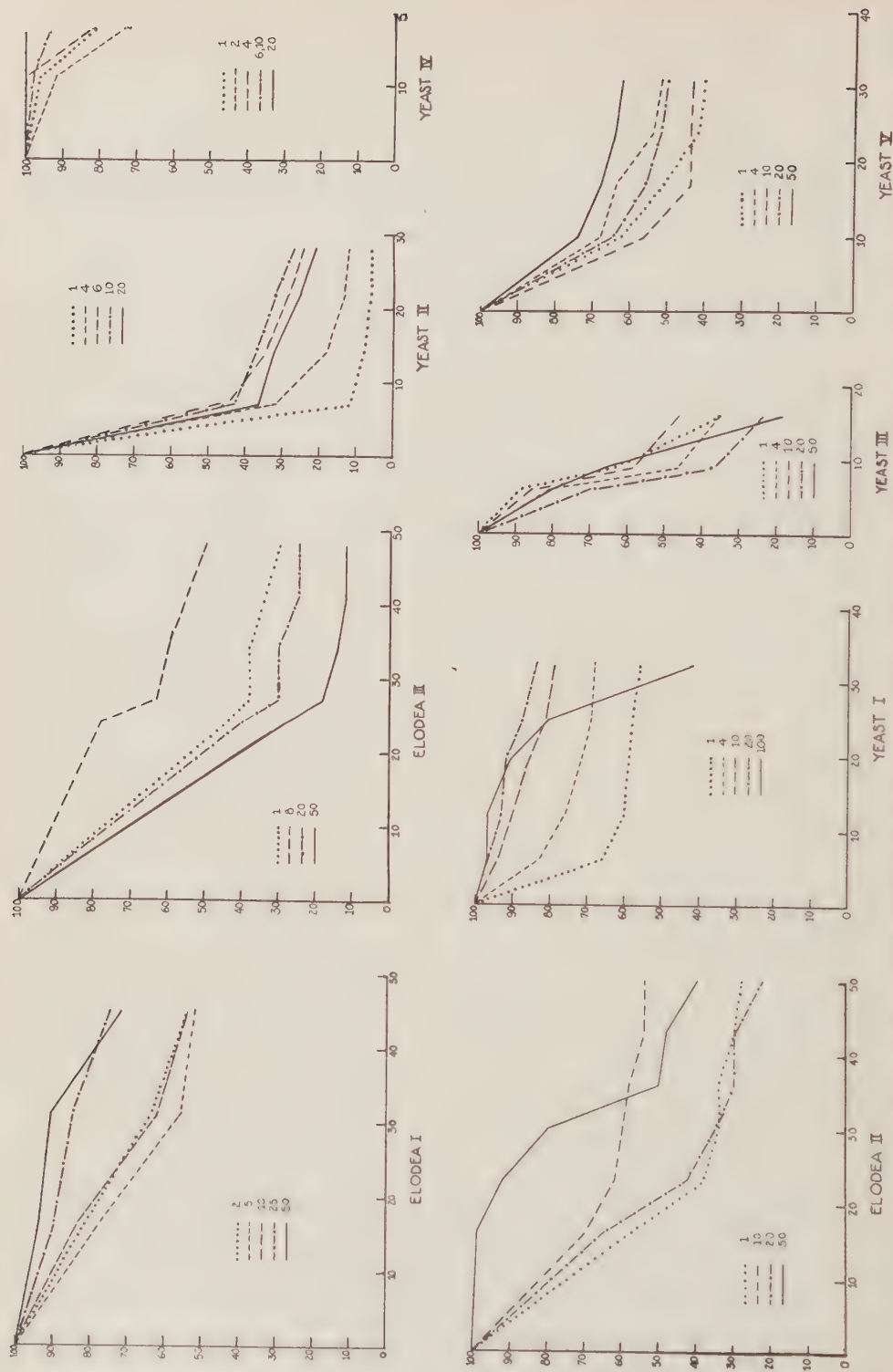


FIG. 5.—Percentage survival in the eight experiments. Ordinates, percentage alive; abscissae, time in days of age; inset key, population density plotted according to given symbols.

lowing experiments. Four days after the experiment had started, the deaths had been so numerous that replacement of animals had been made from the same stock of young originally used. In no other experiment had this been done; the results obtained may be influenced by that method.

In Yeast IV and V the highest density used—20 and 50, respectively—survived the best from beginning to end (Fig. 5), and no shift to a lower density occurred. Because of the use of chloretone, the measure of survival was restricted to 2 weeks and 1 month, respectively, in the two experiments.

In conclusion of this discussion of the effects of density upon the survival of the populations, it may be said that, although survival varied greatly from experiment to experiment and within experiments, nevertheless, a certain pattern in relation to density has emerged in nearly all cases. The type of food and water has not affected the results to an appreciable extent. With one exception (Yeast III, 6 days of age), maximal survival occurred in densities of 10 or above at every measured age. In respect to time the pattern tended to display a shift of survival from a high density to a lower (above 6), with the greatest death rate among the isolated animals and lowest densities in the first week or so of age, after which the death rate rose rapidly in the highest densities.

ENVIRONMENTAL FACTORS IN THE AMPHIPOD POPULATIONS AND DISCUSSION OF THEIR POSSIBLE EFFECTS

Increase in population density has both augmented and decreased the magnitude of physiological response in *H. azteca*. The effect has been dependent upon the particular process considered, the intensity of crowding, and, apparently, to some extent upon the conditions of the environment apart from density.

Density is a highly complex environmental factor and brings concomitant with it Smith's "density-dependent" factors of resistance (Bodenheimer, 1938), or, as expressed by MacLagen and Dunn (1936) "autobiotic factors of control." In the artificial microcosm of the experimental bottles containing the *Hyalella* populations these include directly proportional changes of carbon dioxide tension, other excretory products, fecal matter, collision frequency, and inversely proportional changes in oxygen, food, and space. Some of these have been measured (Table 6). The chemical analyses were made on water from populations seeded with adult males fed upon 4 drops of yeast. The tests started on the sixth day after seeding and ended on the eighth. No attempt has been made to measure the food consumed, but from the original distribution both food and space per animal may be estimated. Densities 50 and 100 seemed to clean up the yeast before the end of the week between feedings; however, there was apparently an excess of food at all times for the isolated animals. The 2 inches of *Elodea* appeared to provide an excess of food for all populations. However, it is realized that a quantitative excess may be qualitatively inadequate.

In Table 6 it may be seen that the 6 measured factors of the environment show a rough proportionality to the density with the exception of pH and conductivity. It is probable that the slight change in pH would have no effect, for the range is far less than that found to be effective on other organisms. The observation that both pH and conductivity dropped very slightly from density 1 to density 4 and then rose again is probably without significance, except in the fact that the two measures support each other. If significant, no explanation is at hand, or for the sudden increase in conductivity at density 50.

The question arises how these measured differentials, ingredients of the "density-dependent environment," may affect physiological processes in arthropods and whether these effects may separately or together have acted to produce the observed results on growth, reproduction, and survival of *H. azteca*.

The retardative effects of quantitative and qualitative malnutrition are too well established to need citation. In fact, von Dehn (1937) considered brood size an indication of the adequacy of the diet fed to cladocerans. In this respect the higher fecundity of the amphipods when fed throughout life upon *Elodea* as opposed to yeast is instructive, quite apart from the question of the population density. Using different diets Anderson (1930)

TABLE 6
ENVIRONMENTAL DIFFERENTIALS DEPENDENT UPON
THE POPULATION DENSITY

Density	CO ₂ * (Cc.)	O ₂ † (Cc.)	Conduc- tivity Mhos × 10 ⁻⁴	pH	Drops of Yeast per Animal	Space per Animal (Cc.)
1.....	52.2	5.10	2.97	7.67	4	100
2.....	52.6	4.96	2.97	7.60	2	50
4.....	52.7	4.87	2.96	7.57	1	25
6.....	53.1	4.67	2.97	7.60	0.67	16.7
10.....	54.2	4.28	3.01	7.70	0.40	10.0
20.....	57.4	4.06	3.09	7.70	0.20	5.0
50.....	66.2	3.70	3.71	7.67	0.08	2.0
Blank 1....	49.8	5.61	2.75	7.83	100 cc. well water 7 days old	
Blank 2....	52.5	5.53	2.85	7.80	The same with 4 drops of yeast	
Blank 3....	55.3	3.95	2.96	7.63	The same with 8 drops of yeast	

* Cubic centimeters CO₂ included that given off by animals, atmospheric, and chiefly that derived from the breakdown of all carbonates and bicarbonates in the water. For the sum of the latter two factors see Blank 1.

† Both gas analyses were made by the Van Slyke method, values are the average of two readings, on the sixth and seventh days, respectively.

found that on a poor diet isolated female *Daphnia* matured later and passed through more instars prior to the release of young. Sexual maturity occurred, he stated, at a given body size rather than at a constant instar. The irregularity of sexual maturity of the isolated control amphipods fed upon a pure yeast diet may correlate with this observation. However, it is in contradiction to results from the experimental populations in which the sexually mature females of the higher population densities (less food per animal) were smaller in size and of a lower antennal segment number than those in less crowded densities (Fig. 4). A lipoid factor, resembling vitamin E, appears to be necessary for maximal reproductive expression in *Gammarus* (O'Brien and Yarnold, 1937). The role of undernutrition associated with crowding was said to account in part for the decreased fecundity of *Drosophila* since many flies disturbed one another while feeding (Pearl, 1932).

The factor of restricted space decreased fecundity of lepidopterans, but the effect was less drastic than that of undernutrition (Hofmann, 1933). Space itself is not a simple factor but involves proportional changes in other factors and cannot, therefore, be used

per se without very careful analysis, despite attempts to consider it of psychological importance.

The increase in some unstable excretory products coincident with crowding is believed to cause the production of males in cladoceran cultures. By this result a depressant action is ascribed to these substances, since artificial depression of metabolism by chemical agents or by low temperature occasioned the appearance of males under conditions otherwise suited to the production of female offspring (Banta, 1937). Carbon dioxide alone did not increase male production in Cladocera (Banta, 1937), and, on the whole, there are few data concerning its action within normal range. In the tests on water from amphipod populations no distinction was made between free and bound carbon dioxide, and it is only the former which would be effective. If pH is an indication of free carbon dioxide, there is much less differential throughout the populations than the analysis for the gas would suggest. Oxygen tension might be effective in producing a proportional metabolic rate if oxygen consumption was dependent upon oxygen tension. This is, however, rarely the case over relatively normal fluctuations of tension. The results on studies of Crustacea allow no generalization (Hyman, 1929).

On the whole, from the few examples given above it can be concluded that at least undernutrition and accumulating excretory products might be responsible for the decrease of fecundity and development at and beyond a density threshold, of which the level would be determined by the susceptibility of the particular process and physiological condition of the organism. At extreme densities one or both might increase the death rate. The rapid death rate of the 100 population at an advanced age (therefore size) could be so explained. The low survival of populations 20 and 50 in *Elodea* III (Fig. 5) when the water was never changed support this view.

No evidence has thus far been presented in explanation of the continued maximal survival and early maximal growth of animals in intermediate densities. Survival at least may be related by analogy to the greater resistance of animals of lowered metabolic rate to adverse elements in the environment in accordance with the work of Child (Fowler, 1931). Evidence is also present that massed animals or their products may ameliorate a toxic or deficient environment (Allee, 1931; 1938). Such an explanation may also be applied to the results on growth and fecundity, with the added complication that in this case the yeast diet must be partially deficient, for the animals fed upon *Elodea* grew inversely to the density, while only those fed upon yeast displayed a positive density effect. The diminution of this effect on growth with increasing age may be related to the increasing strength of the density-dependent factors of resistance as the animals become larger.

Allee (1938) presents the retardative and augmentative effects of density in two curves in order to avoid the use of the terms "harmful" and "beneficial." Magnitude of physiological response is plotted on the ordinates and numbers of animals on the abscissae. The first curve (Type A) is monophasic, showing an inverse relationship; Type B is diphasic with an initially direct relationship which changes to an inverse one. The former curve could be entirely the result of density-dependent factors of resistance which increase in direct relation to crowding. The diphasic curve could be produced by the interaction of two sets of resistances, one of which would act inversely, the other directly in proportion to the density. A toxic or inadequate medium which could be ameliorated or neutralized by the products of density might be considered a resistance inverse to density, for it would retard most strongly the isolated animals. The same products of density increasing beyond the point of neutralization would form the second set of resistances, acting direct-

ly in relation to numbers of animals present. These resistances, whether they act directly with or inversely to density, are examples of density-dependent factors. At the population density in which the sum of the two sets of resistances is least, maximal physiological response would be expressed.

SUMMARY

1. *Hyalella azteca* Saussure has been used to demonstrate the effects of population density upon growth, reproduction, and survival. All populations were contained in 100 cc. of either city water (Lake Michigan), with or without chlorine, or well water and fed upon equal amounts of *Elodea* stem or yeast. The experimental animals were grown under these conditions from a few days after release from the maternal brood pouch until at least 1 month of age. The densities used ranged from 1 to 100 amphipods.

2. When fed upon *Elodea* the animals grew, at least after the second week of age, inversely to the density of the population. The adult females were of a body length inverse to density, as were those fed upon yeast supplemented after 1 month by *Elodea*. In populations fed upon yeast, on the other hand, mean maximal body or head length occurred initially in the animals of a high density, but with increasing age (or size) the maximum shifted to lower densities. Sexually mature males of density 4 were the largest, but the mean body length of females, at the time of first oviposition, was greatest in the isolated animals.

3. On a diet including *Elodea* fecundity appeared to be little affected by density until 6 or more animals were present; beyond this density fecundity was inverse to numbers present. However, on a yeast diet the females of population density 2 were significantly more fecund than those isolated, and the females of population density 4 when fed either yeast or yeast and *Elodea* released the first brood earlier than those from more or from less crowded populations.

4. Survival of the animals varied greatly from experiment to experiment and also in respect to density. The greater survival, however, of animals in populations of 10 or over was substantiated in all eight experiments, and was not related, in any apparent way, to either the type of water or the type of food. As in the results upon growth of yeast-fed populations, an initially maximal survival in a high density (100 or 50) tended to drop to a lower density, but never below 8, with advancing age. This shift was occasioned by a late but very rapid death rate in the highest densities. In the isolated animals or low densities there was initially a high death rate, which decreased as the animals grew older.

5. Analyses of the water containing populations of 1-50 adult males demonstrated a direct proportionality between density and carbon dioxide tension, and an inverse relation between oxygen tension, food, and space. There was little effect on pH. Conductivity changed only slightly until a density of 50 showed a disproportionately great increase.

6. The above stated relation between density and carbon dioxide, presumably other excretory products, food, and space might well be responsible for inhibition of growth and fecundity and, at very high densities, of the ability to survive. It is suggested that the accelerated growth and fecundity of yeast-fed animals and the greater resistance of all animals in an intermediate density may be related to the heightened resistance caused by lowered metabolism or to the amelioration of a toxic or inadequate environment by the coincident products of density.

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